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Respectfully submitted,

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APPENDIX

Version with markings to show changes made

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IN THE SPECIFICATION:

Paragraph 0040 on page 15 has been amended as follows:

--As disclosed herein, the propensity of the non-oligomerizing fluorescent proteins of the invention to oligomerize is reduced or eliminated ~~eliminate~~. In one embodiment, the propensity of a non-oligomerizing fluorescent protein to oligomerize is reduced or eliminated due to operatively linking a first monomer of a fluorescent protein to at least a second monomer of the fluorescent protein, thereby forming an intramolecular 'dimer', 'trimer' or the like. Such operatively linked homopolymers, which are referred to herein as "non-oligomerizing tandem dimers," have a substantially reduced ability to form intermolecular oligomers. Such non-oligomerizing tandem fluorescent proteins are exemplified herein by two monomers of DsRed (SEQ ID NO: 12) operatively linked by a peptide linker (SEQ ID NO: 26), and by two monomers of a mutant DsRed, which has an amino acid sequence of SEQ ID NO: 12, and including an I125R mutation, operatively linked by the peptide linker of SEQ ID NO: 26.--

Paragraph 0048 on pages 17-18 has been amended as follows:

--Another limitation is that, while GFP variants with blue, cyan, and yellowish green emissions have been engineered, all have emission maxima shorter than 529 nm. Recently, polynucleotides encoding six anthozoan (coral) fluorescent proteins having 26% to 30% identity to *Aequorea* GFP (SEQ ID NO: 2) were cloned by Matz et al. (Nature Biotechnol. 17:969-973, 1999, which is incorporated herein by reference). Although most of the coral fluorescent proteins had emission maxima within the range covered by GFP or its variants, one coral protein, drFP583 ("DsRed"; SEQ ID NO: 12), which was isolated from a red portion of a *Discosoma* species, had excitation and emission maxima at 558 and 583 nm, respectively, the longest yet reported for a wild type spontaneously fluorescent protein (Matz et al., *supra*, 1999). Despite the relatively modest sequence identity to GFP, enough sequence similarity was conserved to suggest that the coral proteins would form 11-stranded  $\beta$ -barrels  ~~$\alpha$ -barrels~~, similar to that of

GFP. In addition, the two important residues contributing to the chromophore of GFP, Tyr66 and Gly67, and some of the important polar residues contacting the chromophore such as Arg96 and Glu222, were conserved in the coral proteins. In DsRed, the amino acids corresponding to these GFP residues are numbered Tyr67, Gly68, Arg95, and Glu215, respectively, and additional amino acids that can be involved in oligomerization can be identified using X-ray crystallography methods (see Example 3).--

Paragraph 0085 on page 35 has been amended as follows:

--A variety of *Aequorea* GFP-related fluorescent proteins having useful excitation and emission spectra have been engineered by modifying the amino acid sequence of a naturally occurring GFP from *A. victoria* (see Prasher et al., Gene 111:229-233, 1992; Heim et al., Proc. Natl. Acad. Sci., USA 91:12501-12504, 1994; U.S. Serial No. 08/337,915, filed Nov. 10, 1994, now U.S. Patent No. 5,625,048; International application PCT/US95/14692, now published PCT WO96/23810, each of which is incorporated herein by reference). As used herein, reference to a "related fluorescent protein" refers to a fluorescent protein that has a substantially identical amino acid sequence when compared to a reference fluorescent protein. In general, a related fluorescent protein, when compared to the reference fluorescent protein sequence, has a contiguous sequence of at least about 150 amino acids that shares at least about 85% sequence identity with the reference fluorescent protein, and particularly has a contiguous sequence of at least about 200 amino acids that shares at least about 95% sequence identity with the reference fluorescent protein. Thus, reference is made herein to an "*Aequorea*-related fluorescent protein" or to a "GFP-related fluorescent protein," which is exemplified by the various spectral variants and GFP mutants that have amino acid sequences that are substantially identical to *A. victoria* GFP (SEQ ID NO: 2), to a "*Discosoma*-related fluorescent protein" or a "DsRed-related fluorescent related protein," which is exemplified by the various mutants that have amino acid sequences substantially identical to that of DsRed (SEQ ID NO: 12), and the like, for example, a *Renilla*-related fluorescent protein or a *Phialidium*-related fluorescent protein.--

Paragraph 0089 on pages 36-37 has been amended as follows:

--The term "loop domain" refers to an amino acid sequence of an *Aequorea*-related fluorescent protein that connects the amino acids involved in the secondary structure of the

eleven strands of the  $\beta$ -barrel ~~9-barrel~~ or the central I-helix (residues 56-72). The term "fluorescent protein moiety," when used in reference to a fluorescent protein, refers to a portion of the amino acid sequence of the fluorescent protein that, when the amino acid sequence of the fluorescent protein substrate is optimally aligned with the amino acid sequence of a naturally occurring fluorescent protein, lies between the amino terminal and carboxy terminal amino acids, inclusive, of the amino acid sequence of the naturally occurring fluorescent protein, and comprises a chromophore, which fluoresces upon exposure to an appropriate wavelength of light.--

Paragraph 0098 on page 40 has been amended as follows:

--In one embodiment, each of the first fluorescent protein and the second fluorescent protein is a non-oligomerizing tandem fluorescent protein in a tandem non-oligomerizing fluorescent protein of the invention. For example, the non-oligomerizing tandem fluorescent protein can comprise two or more *Discosoma* RFPs or a fluorescent protein related to a *Discosoma* RFP, such as a DsRed protein having an amino acid sequence as set forth in SEQ ID NO: 12 or a mutant DsRed protein such as SEQ ID NO: 12 containing an I125R mutation. In another embodiment, the first fluorescent protein is a non-oligomerizing tandem fluorescent protein, and the second fluorescent protein is a non-oligomerizing fluorescent protein. The non-oligomerizing fluorescent protein can contain a mutation of an amino acid residue corresponding to A206, L221, F223, or a combination thereof of SEQ ID NO: 2, for example, a mutation corresponding to S65G/S72A/T203Y/H231L in SEQ ID NO: 2. ~~SEQ ID NO: 2; a mutation corresponding to--~~